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PYTHIUM ROOT ROT OF MILO AND THE DEVELOPMENT OF RESISTANT VARIETIES

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PYTHIUM ROOT ROT OF MILO AND THE DEVELOPMENT OF RESISTANT VARIETIES[1]

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MILO (*Sorghum vulgare* Pers.), one of the grain sorghums, is an important agricultural crop in California. In 1937 and 1938 approximately 145,000 acres were grown in the state; and the ten-year average (1929 to 1938) exceeded 100,000 acres. The annual value is approximately three million dollars and in grain crops is exceeded only by barley, wheat, and rice.

The *Pythium* root rot disease of milo, caused by *Pythium arrhenomanes* Drechs., was first recognized in California in 1935. Several years earlier, isolated cases of injury to milo had been variously attributed to alkali, drought injury, and *Fusarium* root rot. In 1936, however, studies of cultures from diseased plants, together with inoculation trials, revealed that these earlier cases had been caused, in all probability, by the *Pythium* root rot fungus.

This disease was first recognized in Kansas (*4, 5, 6*)[4] in 1926 on the experimental grounds of the Garden City branch of the Agricultural Experiment Station. Since then the disease has become rather widespread in certain parts of Kansas, New Mexico, Texas, Oklahoma, and California. Although no extensive survey has been made to determine its distribution in California, it is clearly a problem in the upper delta regions of Sacramento, Solano, and Yolo counties. Besides the observed heavy infestations there, diseased plants have been received from Colusa, San Joaquin, and Imperial counties.

ECONOMIC IMPORTANCE OF PYTHIUM ROOT ROT IN CALIFORNIA

Pythium root rot is the most serious disease affecting milo. Under usual California conditions it spreads rapidly, and soil infestation soon be-

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[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this bulletin.

comes so severe that milo cannot be grown on such soil. In certain delta areas of the Sacramento River, complete killing has resulted the third year after the disease was first observed. The first year, usually, a few isolated, slightly diseased spots appear in a field. Second-year planting to milo shows an increased number of infection centers, many greatly enlarged, and much more severe stages of the disease. Planting in such fields the third successive year invariably results in complete killing rather early in the season. In 1936 the crop in one field of more than 100 acres was completely killed in six weeks to two months after planting and was plowed under. During the season of 1937, hundreds of acres within the known infested areas were plowed under or were not harvested because of this disease. In 1938 the situation was still more serious: the yield on large acreages not previously known to be diseased was reduced from a trace to more than 50 per cent. Thousands of acres are known to be so severely infested that common milo cannot be grown. Because of the sudden appearance of the root rot and its destructive effect, susceptible varieties of milo cannot safely be grown in areas where the disease is known to exist.

THE CAUSAL ORGANISM OF MILO ROOT ROT

As Elliott and others (*6*) have shown, the cause of a milo root rot in Kansas and other southwestern states is a soil-borne fungus, *Pythium arrhenomanes* Drechs. Species of *Pythium* have been associated with root rots of wheat and other cereals in Canada (*11*), of corn in the middle-western states (*1, 7, 10*) and in the Philippine Islands (*9*), and of sugar cane in Louisiana (*3*) and Hawaii (*2*). Comparative studies of these various types of *Pythium* by Rands and Dopp (*8*) have shown that despite some morphological and physiological differences, certain constant distinctive characteristics of the sexual organs warrant classifying them under the one species, *P. arrhenomanes.*

Early attempts to isolate the causal agent of the milo root rot from field-grown plants in California invariably resulted in securing cultures of a *Fusarium.* Preliminary inoculation experiments under controlled conditions showed the *Fusarium* species to be nonpathogenic.

Soil from infested fields was brought into the greenhouse and planted with common milo. The resultant seedlings showed typical root-rot symptoms within two to three weeks after emergence. Tissue plantings on potato dextrose agar plates from the infected roots and crowns of these diseased seedlings readily yielded cultures of a *Pythium.* Subsequent attempts to isolate the causal fungus from severely diseased field material have been difficult because secondary invaders, such as *Fusarium*

species and saprophytic bacteria, usually predominate in the cultures. Where there is some question as to the identity of the disease, the most satisfactory results have been obtained by bringing soil from the field into the greenhouse and growing a susceptible variety of milo in it. The typical symptoms appear in the seedling stage, and the pathogen is easily isolated.

EXPERIMENTS IN SOIL INOCULATION

Sixteen *Pythium* isolations from four different locations in California were used for a series of inoculation experiments in the greenhouse. A culture of *Pythium arrhenomanes* from diseased milo was furnished by Dr. C. L. Lefebvre of the Kansas State College, and two cultures of the same fungus from rootlets were secured through the courtesy of Dr. Charlotte Elliott of the United States Department of Agriculture Bureau of Plant Industry.

The cultures from California, from Kansas State College, and from the Bureau of Plant Industry were grown either on agar plates or on steamed oats in small flasks and were added to 6- and 8-inch pots of steam-sterilized soil. In each pot were planted approximately fifty seeds of Double Dwarf Yellow milo, while a comparable series of pots of sterilized, uninoculated soil were planted as controls.

In the first series of trials most of the plants were killed before, or just after, emerging in the inoculated pots. The extreme severity of the disease in this series was attributed to the fact that the seeds were placed close to the infective material.

In a second series of trials with the same pots, the top 1 to 2 inches of infested soil was removed, and a thin layer of sterile soil added. The seeds were placed on the layer of sterile soil and covered with an additional layer of steam-sterilized soil. In this second series the seeds germinated normally, and the disease was somewhat delayed and less severe.

Typical symptoms of the root rot, such as occur in the field or in infested soil brought into the greenhouse, showed within one month after planting. Many plants were entirely killed, and the others showed severe stunting and yellowing of the leaves. Examination revealed an almost complete rotting of the root system and a characteristic brick-red discoloration of the inner crown tissue.

In both series of experiments the culture from Kansas failed to produce the disease, whereas the culture from Washington, D. C., produced symptoms identical with those produced by the California strains of the organism. In all cases, the plants in the control pots were vigorous and healthy.

TESTS TO SHOW THE PRESENCE OF THE CAUSAL FUNGUS IN THE SOIL

In order further to determine whether the root rot of milo in California was the same as the root rot in Kansas, soil from around diseased field plants was sent to the Kansas State College in August, 1936, for comparative greenhouse trials. On November 11, 1936, a letter from Professor L. E. Melchers of that college contained the following passage:

> The test with the California soil was completed sometime ago, and there is no question of doubt but that your soil contains the same organism which is causing our milo disease.
>
> Dr. Lefebvre has examined some of the material, and *Pythium arrhenomanes* is there in abundance. The resistant strain of Dwarf Yellow milo stood up one hundred per cent on the California soil, and the ordinary Dwarf Yellow milo died one hundred per cent.

In August, 1936, seed of root-rot-resistant Dwarf Yellow and Wheatland milos was received from Mr. F. A. Wagner of the Garden City Branch of the Kansas Agricultural Experiment Station. This seed was used in a field to plant a small plot (75×80 feet) in a place where 100 per cent of the plants of Dwarf White milo had been killed during the current season. A total of 12 rows of resistant Dwarf Yellow, 16 rows of resistant Wheatland, and 22 rows of Dwarf White were planted at random in the plot. On October 7, 1936, all plants in the 22 rows of Dwarf White milo were greatly stunted, yellow, and dying, whereas the resistant types were thriving, with no evidence of the root rot.

Although there has been no critical study of the species of *Pythium* affecting milo in California as compared with a similar disease in Kansas and southwestern states, the preliminary studies at this station and those of Melchers indicate that the causal agent is the same.

SPREAD OF THE DISEASE BY SOIL AND WATER

Under California conditions the disease apparently spreads rapidly from field to field and from one locality to another. As Elliott and others (*6*) have shown, the disease is readily transmitted by soil, diseased plant refuse, and soil water. Greenhouse trials under controlled conditions at this station have proved that transmission by the soil is readily possible. Judging from observational evidence under field conditions, irrigation water, as well as overflow and runoff water, is probably the most common method of spread in California. Elliott and others (*6*) found no evidence of transmission by seed. During the winter of 1936–37, the present authors planted seed from severely diseased plants in steam-sterilized

soil. There was no evidence of the disease on the 587 plants grown in this test. The transfer of soil from an infested to a noninfested area by farm equipment or by other means is an important method of spread.

PLANT ROOTS ATTACKED BY THE FUNGUS

The first evidence of the disease is on the aboveground parts of the plants when they are 4 to 6 inches high, usually 4 to 6 weeks after planting (fig. 4). The young plants show stunting, a slight rolling of the leaves,

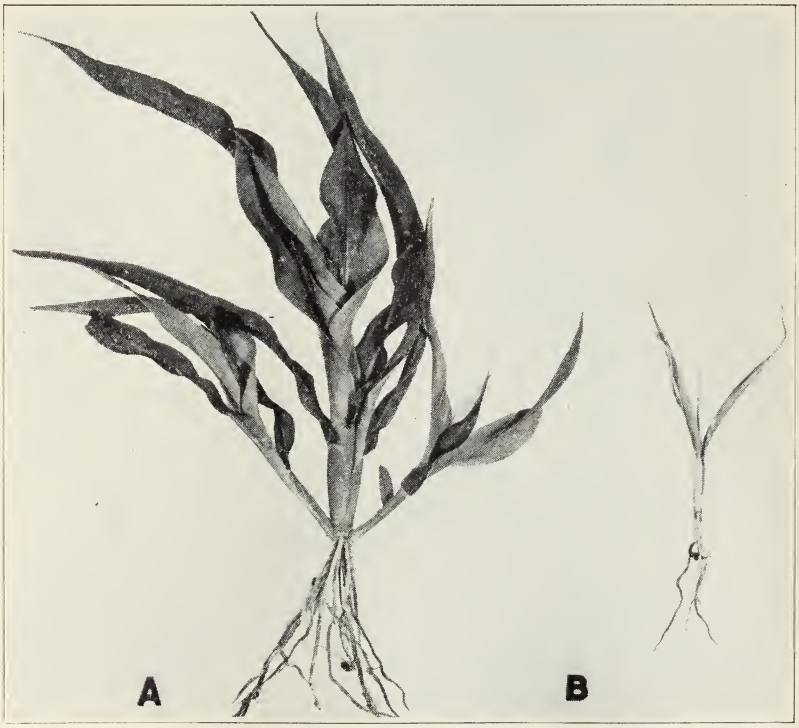

Fig. 1.—Plants of milo 33 days after planting. *A*, Resistant to *Pythium* root rot; *B*, susceptible. (Both one-half natural size.)

and a slight reddish-yellowing of the margins and tips of the first leaves (fig. 1, *B* and fig. 2). In heavily infested soil the disease, as evidenced by the reddish-yellow color and death of the tissue, progresses rapidly until the entire plant is killed. In soils less heavily infested, the disease may not appear until the plants are 8 to 12 inches high or until midsummer; and its progress is less rapid. In such cases the plants may continue a weak growth until late in the season; and sometimes they produce weak heads, which may contain a few small grains (fig. 6).

Although onset and effect are rapid and severe in heavily infested soil, in less heavily infested soil or in first-year infection the disease is first evidenced by irregular spots or areas containing somewhat stunted pale-

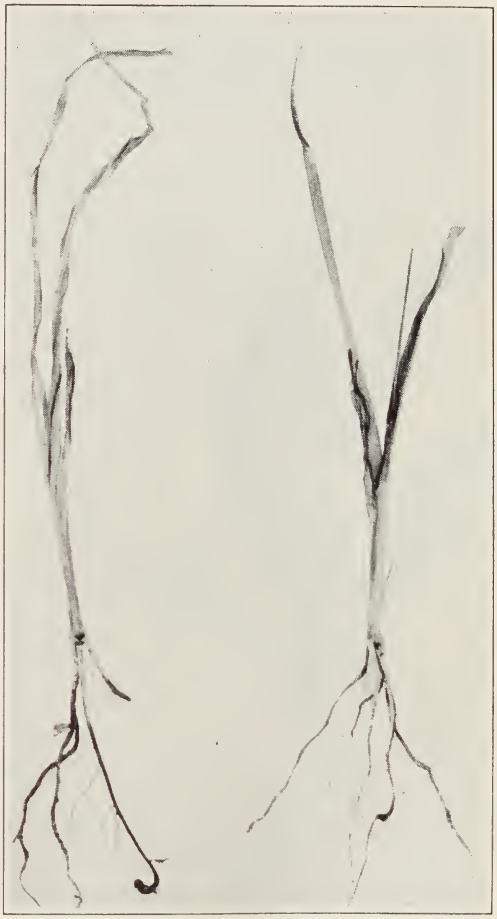

Fig. 2.—Common Double Dwarf Yellow seedling milo plants showing severe injury from *Pythium* root rot. Note the reddish color on the margins and tips of the leaves on the plant to the left, as represented by the darker color. The roots also showed a red coloration.

green to dull-yellowish plants, resembling areas of drought or alkali injury.

Shortly before the appearance of the symptoms on the leaves, the roots show a water-soaked brown condition of the cortex and vascular system.

Secondary rot-producing organisms soon destroy most of the fine roots. The cortex of the larger roots sloughs off, leaving the central cylinder as a brown or dark-red hardened body. An easily recognized and reliable

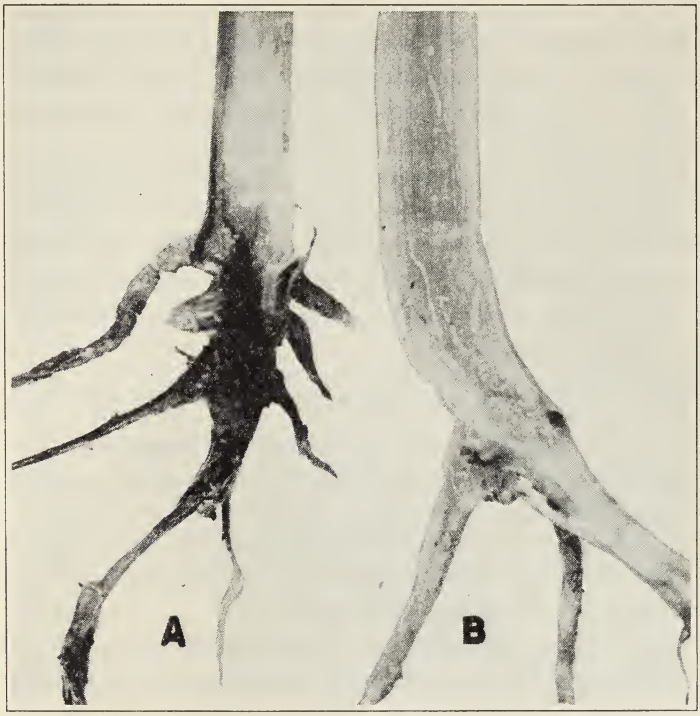

Fig. 3.—Longitudinal sections of Double Dwarf Yellow milo plants. *A*, Plant affected with *Pythium* root rot, showing the discoloration of the inner crown tissue and main root and the sloughing of the cortex of the lateral roots. In the fresh plant this discoloration is dark red. *B*, Healthy plant showing the absence of the discoloration and sloughing of the root cortex. (× 6.)

symptom is the wedge-shaped dark-red discoloration of the internal tissue at the base of the crown, which is shown as a dark area on the plant at *A* in figure 3.

This discoloration often extends for a short distance up through the vascular system of the stalk and into the larger roots emanating from the crown. The effect of drought injury on the aboveground part of the plants closely resembles the mild stages of root rot, but the two can usually be differentiated by cutting through the lower crown tissue. A further point of difference is that the dark-red color is absent in the tissue of drought-injured plants.

VARIETIES RESISTANT TO PYTHIUM ROOT ROT

According to Elliott and others (*6*) and Wagner (*12*), the disease cannot be controlled by any practical method of seed or soil treatment. As Wagner (*12*) has also shown, kafirs, feteritas, and sweet sorghums are highly resistant, whereas milos and most milo derivatives are very susceptible. Resistant strains, however, of Dwarf Yellow (the Finney strain), Wheatland, and Beaver types of milo developed by Wagner (*12*) are now being grown commercially with a high degree of resistance.

Judging from trials, the resistant Finney and Wheatland types of milo in California are also highly resistant to root rot. The resistant Finney is being grown to some extent commercially in infested areas. On most California soils the Standard Dwarf types of milo grow rather tall, often show severe lodging, and are less adaptable to combine harvesting than the Double Dwarf varieties.

INFESTED FIELD SOIL USED IN GREENHOUSE STUDIES

In the late summer of 1936, considerable soil from severely diseased areas in five localities was brought into the greenhouse and placed either in greenhouse benches or in large pots; in each case, common Double Dwarf Yellow milo was grown in the soil to determine the presence of the disease. All soils showed a heavy infestation, nearly all of the plants being killed within a few weeks after planting.

In this heavily infested soil a planting was then made to determine the susceptibility of several varieties of grain sorghums and Sudan grass. The following varieties were planted: White durra, Spur feterita, Hegari, Dwarf Hegari, Black Hull kafir, Dawn kafir, Pink kafir, Dwarf Yellow milo (Heileman), Double Dwarf Yellow milo, Dwarf White milo, Fargo, Kaoling, Shallu, Darso, Dwarf Darlo, Double Dwarf Darlo, Sumac sorgo, Atlas sorgo, Black Amber sorgo, and Sudan grass. All these proved highly resistant except Darso, Double Dwarf Yellow, Dwarf White, and Heileman milos, which showed serious susceptibility. A similar trial in the same soil in 1937 yielded the same results.

RESISTANT PLANTS FOUND IN COMMERCIAL FIELDS

During the summer of 1936, in fields where most of the plants were dead from root rot, usually a few plants appeared normal in every respect. These, when more closely examined, evidenced high resistance to the disease. In two fields of Double Dwarf Yellow milo planted with Calapproved seed stock on Ryer and Prospect islands, seventeen apparently resistant plants were harvested separately. Also in the Delta area, six

surviving plants of Dwarf White milo in a severely diseased field were harvested individually in an effort to develop resistant strains.

Double Dwarf Darlo, a New Resistant Variety.—In the greenhouse susceptibility trials Double Dwarf Darlo, a new variety of milo developed at this station by Mr. L. G. Goar of the Division of Agronomy, was among the varieties that proved highly resistant. From a cross of Darso X Heileman (Dwarf Yellow) milo, Goar selected a strain that was named Darlo. Because it was too tall for combine harvesting, he crossed it with Double Dwarf Yellow milo. From this cross Double Dwarf Darlo was selected. Judging from results on experimental plots at Davis, it is equal to Double Dwarf Yellow milo in yield. Commercial plantings in the delta of the Sacramento River during the past two years have yielded satisfactorily (fig. 7). In general, Double Dwarf Darlo matures about two weeks later than Double Dwarf Yellow milo—a serious fault in some regions and one that unfits it for late planting or double cropping. The rather limited experience with Double Dwarf Darlo indicates, furthermore, that it will not stand such adverse conditions as alkali, drought, and extreme heat so well as Double Dwarf milo. It has, however, served a useful purpose in the delta of the Sacramento River, producing as much as 6,500 pounds of grain on large acreages where Double Dwarf Yellow milo would have been a complete failure because of *Pythium* root rot.

Tests with Single-Plant Selections.—Since previous trials with susceptible varieties and varietal tests in the infested field soil brought into the greenhouse showed that the disease developed normally and rather quickly under these conditions, the seed from the single plant selections of Double Dwarf Yellow, Dwarf White, and the Double Dwarf Darlo variety was planted in duplicate in this soil. In repeated tests during the winter of 1936–37, susceptible Double Dwarf Yellow was used as a control. The results are summarized in table 1.

Field Trials of Resistant Strains.—To test further these apparently resistant strains of milo under field conditions, approximately 4 acres were selected in 1937 for a plot in a field on Ryer Island where milo had been completely killed in the seedling stage by *Pythium* root rot in 1936. In this field plot, thirteen selections of Double Dwarf Yellow and six selections of Dwarf White, together with Double Dwarf Darlo, resistant Finney milo, and resistant Wheatland types, were planted. The two last-mentioned varieties are not given in table 1. Each alternate row was planted with common Double Dwarf Yellow for a control. The rows were approximately 400 feet long, and each selection and type was replicated three times.

One month after May 14, the date of planting, the first symptoms were

showing in the control rows. On July 3, or six weeks from planting, the control rows showed practically 100 per cent dead plants. In most of the rows planted with the single plant selections, very few diseased plants appeared. Counts were made in replication number 1, but were not taken

TABLE 1

SUMMARY OF TRIALS OF SINGLE-PLANT SELECTIONS OF VARIOUS MILO VARIETIES FOR SUSCEPTIBILITY TO *Pythium* ROOT ROT IN NATURALLY INFESTED SOIL IN THE GREENHOUSE AND IN AN INFESTED FIELD PLOT

Selection No.	Variety	Greenhouse trials in 1936-37			Field trials in 1937	
		Number of trials	Total number of plants	Per cent diseased	Total number of plants	Per cent diseased
1	Double Dwarf Yellow	13	542	8.7	333	0.3
2		13	444	6.7	233	0.4
3		13	559	4.4	337	0.9
4		13	471	6.1	236	0.4
5		13	548	9.6	232	1.2
7		13	607	11.2	358	1.4
8		13	685	4.3	387	1.3
9		13	525	5.7	248	0.0
10		13	665	7.2	368	3.8
11		11	576	7.6	407	6.8
30		4	159	6.6	319	1.8
31		4	167	1.1	341	1.7
32		4	155	3.8	212	3.8
14	Dwarf White	11	494	13.0	363	7.7
15		8	318	9.1	340	10.0
16		8	302	5.6	298	2.6
17		8	306	6.5	309	0.9
18		5	182	21.4	307	12.0
19		5	182	11.0	261	0.7
..	Double Dwarf Darlo	9	409	5.8	293	0.0
..	Common Double Dwarf Yellow	22	700	96.7	350	100.0

in the other two replications because there was apparently no difference in the amount of disease over the entire planting.

The results (table 1) show that all the single-plant selections manifested high resistance both in the severe greenhouse tests and in the field trials. With one exception, furthermore, the percentage of diseased plants was somewhat higher under greenhouse conditions than in the field. The rapid succession of crops and the favorable water and temperature conditions in the greenhouse trials probably produced an extremely heavy soil infestation.

BAGGING HEADS TO SECURE PURE SEED STOCK

During the summer of 1937, shortly before the blossoming period, approximately 100 heads in each selection were bagged in the field plot to secure selfed lines for further trials. Throughout the season, the different selections were critically examined and compared. In the end there appeared to be little, if any, difference between them as to vigor and type of plants produced. With few exceptions, they were all apparently good, representative types of Double Dwarf Yellow and Dwarf White milo. The seed from the bagged heads, therefore, was harvested in bulk.

INCREASED RESISTANCE SHOWN IN GREENHOUSE AND FIELD TRIALS IN 1938

As a further test, the thirteen resistant strains of the Double Dwarf Yellow, bagged in 1937, were planted in infested greenhouse soil used previously for susceptibility trials. The results showed complete resistance, whereas control plantings of common Double Dwarf Yellow showed 100 per cent susceptibility.

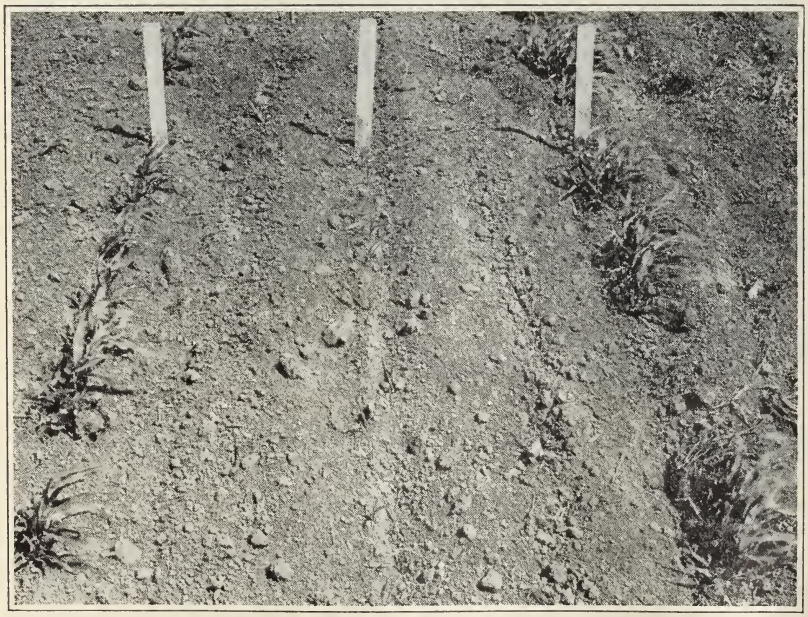

Fig. 4.—Sections of three rows in a field plot of soil infested with *Pythium* root rot photographed 33 days after planting. The outer rows are resistant strains of Double Dwarf Yellow, and the center row is common Double Dwarf Yellow. The center row shows all of the plants killed by the root rot.

In 1938, duplicate plantings of thirteen strains of Double Dwarf Yellow and five strains of Dwarf White were made on the same field plot used in 1937. Double Dwarf Darlo and Finney milo from Kansas were also included. Each alternate row was again planted with common Double Dwarf Yellow milo for a control. The plot was planted on May 2, and on June 2, the control rows showed 100 per cent dead or dying plants

Fig. 5.—View of the experimental plot of soil infested with *Pythium* root rot; Ryer Island, 1938. These are alternate rows of Double Dwarf milo resistant to the root rot shown as they appeared just before harvest. The in-between rows were planted with common Double Dwarf Yellow milo in which the plants were all killed in the seedling stage as shown in figure 4.

(fig. 4). Killing was more rapid than in 1937. The selections of Double Dwarf Yellow and Dwarf White, together with the Double Dwarf Darlo and the Finney milo, again showed complete resistance (fig. 5).

SEED-INCREASE PLANTING FROM BAGGED HEADS

The seed harvested in 1937 from bagged heads of ten of the thirteen resistant strains of the Double Dwarf Yellow selections was bulked for an increase planting in an isolated area in 1938. Throughout the season the planting was carefully rogued for off-type plants and observed as to growth habit; it showed very uniform growth, with relatively few off-type plants, and conformed entirely to the characteristics of the common Double Dwarf Yellow milo. This seed is being distributed through the Calapproved seed plan.

DOUBLE DWARF MILO 38 IS RESISTANT TO PYTHIUM ROOT ROT

Considerable foundation seed resistant to *Pythium* root rot is available for 1939 planting and is being distributed as Double Dwarf Milo 38. In 1939 and thereafter, only this resistant strain of Double Dwarf Yellow milo will be Calapproved.

Fig. 6.—A field of common Double Dwarf Yellow milo showing severe injury from *Pythium* root rot. The plants are yellow, and relatively few heads have been produced; the few heads there are, show as dark spots in the figure.

Seed of Dwarf White milo resistant to root rot is being increased to be released under the Calapproved plan.

Resistant selections have been made from Heileman milo in much the same way as with the Double Dwarf Yellow and Dwarf White selections. The Heileman selections will be given additional field trials for purity and resistance before being increased for distribution.

SUMMARY

Pythium root rot, a destructive disease of milo, has been recognized in the upper delta region of the Sacramento River since 1935. The disease, now widespread, is the limiting factor in milo production in this section where common varieties have been grown.

The disease is caused by a soil-borne fungus, *Pythium arrhenomanes* Drechs, spread from field to field and from area to area by overflow water, irrigation, or any other means whereby diseased soil is transported.

The fungus destroys the roots, causing the plants to die in the seedling stage; or, in less severe attacks, stunts and weakens plant growth.

A varietal greenhouse test showed Double Dwarf Darlo, a new variety developed by the Agronomy Division, to be highly resistant to the disease. On most soils, Double Dwarf Darlo compares favorably with Double

Fig. 7.—A field of Double Dwarf Darlo that is highly resistant to *Pythium* root rot.

Dwarf Yellow milo in yield, but has the disadvantage of being about two weeks later in some sections and less tolerant of alkali and heat than the latter.

Severely diseased commercial fields of Double Dwarf Yellow and Dwarf White milos showed a few apparently normal plants. Single-plant selections of these two varieties were made in severely diseased commercial plantings in 1936.

Repeated greenhouse trials in naturally infested field soil during the winter of 1936–37 showed most of these strains to be highly resistant. The greenhouse results were confirmed by an extensive field trial in severely diseased soil in 1937.

A considerable number of heads were bagged before blooming in the field trials during 1937 to secure pure-line resistant seed.

Further greenhouse trials during the winter of 1937–38 and field trials

in the summer of 1938 showed the selected strains to be practically 100 per cent resistant.

Since the selections of Double Dwarf Yellow milo showed no marked differences in their growth habit or yield, the seed from the bagged heads in 1937 was bulked for increase planting in 1938. Considerable resistant milo seed harvested from this planting is available and is being distributed through the Calapproved plan as Double Dwarf Milo 38.

The resistant strains of Dwarf White and Heileman will be increased for distribution under the same plan.

ACKNOWLEDGMENTS

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